AMD Biotech Inc.
Advanced Molecular Detection

Product Manual

Auto-Mag® Mega-Select

Version 2.2.1

Magnetic Beads-Based Reagent for short DNA fragment depletion and size selection of High Molecular Weight (HMW) DNA sample for Long-Read Sequencing (LRS)

Catalog Number: S005-01, S005-02, S005-03, S005-04

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Disclaimers and Safety Information

This kit is designed for research use only. All biological samples are considered potentially infectious. When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). MSDS can be downloaded from the "Product Documents" tab when viewing the product kit. Download MSDS at www.amdbiotech.com. Information in this document is subject to change without notice.

Product Introduction

Long-Read DNA sequencing platforms, with read lengths >10kb, require high molecular weight DNA; however, purification of such long DNA molecule is difficult and often returns fragments of various sizes, requiring some form of post-extraction cleanup procedure to recovery HMW DNA fragments. The Auto-Mag® Mega-Select provides a quick method to remove unwanted short DNA fragments and recover HMW DNA fragments from DNA samples.

Auto-Mag® Mega-Select consists of AMD Biotech's own paramagnetic beads and compatible buffers. By simply adjusting the volume ratio of reagent to sample, DNA <5kb, <10kb, or <20kb in length can be quickly removed to trace levels from high molecular weight (HMW) DNA samples which leads to the recovery of large DNA fragments and significantly enhance mean read length of third-generation sequencing platforms, such as PacBio SMRT Technology or Oxford Nanopore Technology.

The Auto-Mag® Mega-Select Kit can be used to quickly cleanup unsheared HMW DNA samples and can also be used to selectively recover desired size HMW DNA fragments from fragmented HMW DNA samples or even partially degraded gDNA samples for library preparation of long-read sequencing (LRS). Since Auto-Mag® Mega-Select uses magnetic technology and requires no centrifugation or filtration, it is suitable for manual processing as well as adapting to automatic liquid handling workstations.

Features:

- Works with HMW DNA
- Effective purification and recovery of HMW DNA, dsDNA fragments 5kb or longer.
- Removal of short DNA fragments, impurities, and unwanted reaction components
- Fragment size selection of HMW DNA for specific applications of long-read sequencing.
- Expectant recover Yield: Up to 90%
- Compatible with manual and automated processing and cost effective.

Kit Contents

Product Number	**S005-01**	**S005-02**	**S005-03**	**S005-04**
Preps	25	100	250	1,000
Auto-Mag® Mega Select	2 ml	8 ml	20 ml	80 ml
Elution Buffer	2 ml	8 ml	20 ml	80 ml

Storage and Stability

Auto-Mag® Mega Select is shipped at ambient temperature and is stable for 12 months when stored at 2-8°C. Contents of the kit should never be frozen at any time.

Preparation of Reagents

1. Prepare 80% Ethanol wash buffer. (Prepare from absolute ethanol. Do not use denatured alcohol).

Ethanol is hygroscopic. When opened the ethanol will both evaporate and absorb water over time. Fresh prepare 80% ethanol then keep cover tight and use within one week.

Additional Information

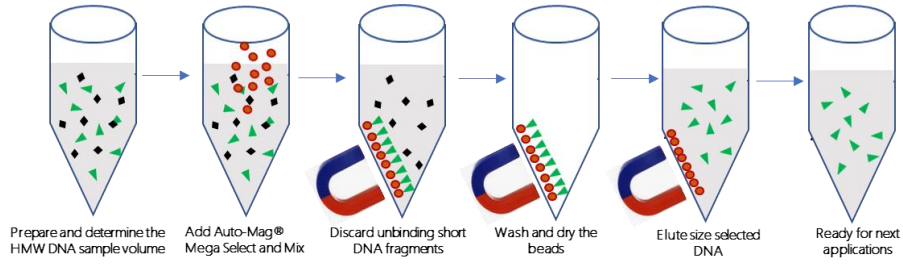
1. Heterogeneity and viscosity

The size selection accuracy and recovery efficiency of the Auto-Mag® Mega Select kit depend in part on the input DNA being homogeneous and fully in solution. HMW gDNA can sometimes be difficult to re-solubilize after extraction and results in an inhomogeneous or contains sticky jellies. Such samples may result in low yields and short DNA carryover. If the HMW gDNA sample is inhomogeneous or contains sticky jellies, we recommend shearing sample with a 26G needle 2-5 times before beginning size selection. Sample homogeneity can be evaluated by performing triplicate concentration measurements and verifying that the concentration CV is <10%.

2. Pipetting

When adding, mixing sample, use wide bore pipette tips. Do not disturb beads when removing liquid from the microcentrifuge tube or well of plate. Carefully insert the pipette tip against the wall to dispense, likewise, remove liquid by pipetting from the liquid surface.

HMW DNA cleanup and size selection process workflow



1. Confirm the Quality and volume of input HMW DNA samples
2. Add Auto-Mag® Mega Select reagent and incubation at room temperature (15-25°C)
3. Bind sample to paramagnetic beads and discard supernatant
4. Wash beads
5. Elute and recovery size selected HMW DNA
6. Transfer eluate to new vessel and ready for next applications.

Auto-Mag® Mega Select Protocols

Required Materials and Equipment

- Single-tube format: 1.5 ml Eppendorf DNA LoBind tube and Magnetic Rack Separator for tube. (Any vendor of choice).
- 96-well format: 96 well thermal cycling plate, or 500µl round bottom microtiter plate, and appropriate magnetic separation device (www.fishersci.com or any vender of choice).
- 200µl wide bore pipette tips (USA Scientific (1011-8410) or any vender of choice.)
- Well calibrated pipettor and Disposable pipette tips.
- Polypropylene reservoirs
- DNA quantification equipment. (Thermo Fisher Scientific NanoDrop 2000. Thermo Qubit 3.0, Qubit dsDNA BR Assay Kit, etc.)
- Laboratory mixer, vortex, or equivalent.
- 80% ethanol (Prepare from absolute ethanol. Do not use denatured alcohol).

Before Starting

- Prepare 80% Ethanol for DNA wash steps.
- Bring the Auto-Mag® Mega Select reagent to room temperature for at least 30 minutes before use.
- Complete suspending Auto-Mag® Mega Select reagent by vortex.
- Check the pipettor and confirm it is well calibrated.

Protocol for Short DNA Fragments Depletion and HMW DNA Size Selection.

This procedure describes the workflow to remove short DNA molecules from unsheared high molecular weight (HMW) genomic DNA, sheared genomic DNA fragments, or partially degraded genomic DNA samples. By adjusting the ratio of reagents to samples, the desired size ranges of HMW DNA fragments can be recovered. The recovered HMW DNA is also cleaned during the process which could help remove contaminants from samples.

Sample Quality Control

Long-read sequencing requires high-quality, high molecular weight (HMW) DNA. Before performing DNA cleanup, it is necessary to evaluate the quantity and size distribution of input DNA to determine whether it is suitable for the protocol.

- To isolate HMW genomic DNA with a qualify HMW DNA isolation kit and 50% of HMW DNA should be >30 kb.

- DNA concentration should be measured using a Qubit dsDNA Broad Range Assay or equivalent and concentration is between 5–150 ng/µl. If the DNA concentration is over 150ng/µl, dilute sample using TE buffer (pH 8), Elution Buffer, or water.

- Unsheared genomic DNA sample should be in DNA Elution buffer, TE buffer (pH 8.0) or water and concentration is between 10–150 ng/µl. If you are unsure of the buffer composition or if the gDNA is not in Elution Buffer, perform a 1X Auto-Mag® X-Pure Select, or AMPure XP bead purification followed by elution with Elution Buffer or an equivalent low salt buffer (i.e., TE Buffer, 10 mM Tris-HCl, pH 8.0, or water)

- The sheared genomic DNA sample should be in low Ionic strength buffers. If not, perform a 1X Auto-Mag® X-Pure Select, or AMPure XP bead purification followed by elution with Elution Buffer or an equivalent low salt buffer (i.e., TE Buffer, 10 mM Tris-Cl, pH 8.0, or water). The concentration of fragmented DNA can be between 5–150 ng/µl.

- For partially degraded genomic DNA samples, DNA samples should first be purified by using 1X Auto-Mag® X-Pure select and eluted in Elution Buffer or an equivalent low salt buffer (i.e., TE Buffer, 10 mM Tris-HCl, pH 8.0, or water). The concentration can be adjusted to 5–150 ng/µl.

- Sample volume: Although the starting sample volume can be any volume greater than 10ul. However, the size range of recovered gDNA is sensitive to changes in the volume ratio of reagent to sample. Using a smaller sample volume may increase the chance of size shift of recovered DNA by sampling error. It is recommended to use 50µl of sample. If a smaller sample volume must be used, please refer to the volume ratio of reagent to sample corresponding to the size range of recovered DNA and use a more accurate pipette.

Protocol

1. Completely float the Auto-Mag® Mega Select reagent until it appears homogeneous in color.

2. Pulse vortex or pipette mix each sample to homogenize the DNA in solution. Adjust the DNA sample to a total volume of 50µl and DNA concentration between 10–150 ng/µl.

   Note: Quick spin each sample to collect liquid if necessary.

3. Pipette 50µl of the sample into a 1.5 ml Eppendorf DNA LoBind tube, or the well of 96 plate (96 well format)

4. For depleting <5kb DNA fragments, add the 75µl of Auto-Mag® Mega Select reagent (1.5x) to the sample.

   Note: for removing other size short DNA, please reference Table 1, Add the appropriate volume of Auto-Mag® Mega Select reagent to the sample.

Table 1. recommended ratio of Auto-Mag® Mega Select to the gDNA sample

for removing short DNA fragments and size selection

Size of short DNA to be removed	*Ratio of Auto-Mag® Mega Select / Sample*	*Auto-Mag® Mega Select Needed (µl) (For 50µl sample)*
< 3 kb	*1.65 x*	*82.5µl*
< 5 kb	***1.5 x***	***75µl***
< 10 kb	*1.45 x*	*72.5µl*
<20kb	*1.35x*	*67.5µl*
<40kb	*1.3-1.25x**	*65-62.5µl**
****** When selecting DNA fragments above 40kd, the concentration and content of DNA above 40kd in the sample may affect the selection results and recovery rate. Sometimes, the ratio of reagent to sample may need to be adjusted slightly to harvest the desired DNA.*		

5. Mix the sample by gently pipetting up and down 15 times with wide bore pipette tips.

   Note: It is important to mix well.

6. Incubate sample at room temperature for 10 minutes at least for maximum recovery.

7. Place the sample tubes or plate on a compatible magnetic separation device for 5 minutes or until the solution clears. Beads will pull to the side of the well.

   Note: The magnetic separation time may relate to the strength of the magnetic separation device and the sample volume. If necessary, gently pipette the liquid up and down twice to speed up magnetic bead separation. Do not touch the beads.

8. With the sample still on the magnet, carefully remove the supernatant by pipetting the liquid out which contains the unwanted smaller DNA fragments. Do not disturb the beads while pipetting out the supernatant.

   Note: The supernatants have unbinding short DNA fragments. If unbound DNA needs to be recovered, transfer supernatant to a new tube or 96-well plate. Reference the protocol of unbinding short DNA fragments to recover the small DNA fragments.

9. With the samples still on the magnet, slowly add 200µl of 80% ethanol to each sample and gently pipet the liquid up and down twice. After incubating 30 seconds, slowly pipetting out and discard all of liquid. Do not touch the beads.

   Note: 80% ethanol is hygroscopic and should be prepared FRESH to achieve optimal results.

10. Repeat steps 9 two more times.

11. Keep the sample on the magnet, and air dry the magnetic beads at room temperature for 3 minutes. Remove any residue liquid with a pipettor.

    Note: It is critical to completely remove all traces of alcohol but take caution in not over drying the beads as this will significantly decrease elution efficiency.

12. Remove the sample from the magnet. Add 20-100µl of Elution buffer. Let the sample sit at room temperature for 5 minutes. Do not suspension and mix the sample at this step.

Note: Elution Buffer volume may be adjusted to achieve desired concentration, but should be large enough so that the liquid level is high enough on the magnet for the beads to settle.

13. After 5 minutes incubation, gently pipetting up and down elution buffer 15 times with wide-bore pipette tips to ensure the beads are properly re-suspended in the elution buffer. Incubate sample again at room temperature for an additional 5 minutes.

    Note: When resuspending magnetic beads, the volume of the pipettes should be adjusted to be smaller than the volume of Elution buffer to prevent bubbles generated when suspending magnetic beads.

14. Place the sample back on the magnet for 5 minutes or until the magnetic beads are completely cleared from solution.

15. Transfer the eluate to an appropriate storage vessel and analyze the recovery and purity of the DNA using a NanoDrop and Qubit system.

16. DNA can be stored in Elution Buffer at 4°C for several months. Keep at -20°C for long term storage. Avoid freeze/thaw cycles since this can degrade high molecular weight DNA.

Supplemental protocol for recovery unbinding short DNA fragments

The following protocol is for recovery all unbinding DNA fragments from supernatant of removing short DNA fragments and size selection procedure by using Auto-Mag® X-Pure select or compatible reagents.

Additional Materials and Equipment:

- Auto-Mag® X-Pure select (Cat: AMD-S003) or compatible reagents.

Before Starting

- Prepare 80% ethanol for wash steps. A minimum of 0.5 ml is required per sample.
- Complete suspending Auto-Mag® X-Pure Select reagent by vortex.

Procedure

1. Confirm the volume of supernatant from Step 8 of removing short DNA fragments and size selection procedure.

2. Add 1x volume of *Auto-Mag*® X-Pure Select reagents to the sample.

3. Mix thoroughly the Auto-Mag® X-Pure Select reagent and sample by pipetting up and down 15 times. Incubate at room temperature for 5 minutes.

4. Place the samples on the magnetic separation device for 3-5 minutes or until the Auto-Mag® X-Pure Select beads are completely cleared from solution. Carefully remove and discard the supernatant.

5. Keep the sample on the magnetic separation device, add 200µl of 80% Ethanol to each sample and incubate for 1 minute at room temperature. Carefully remove and discard the supernatant.

   Note: It is not necessary to resuspend the Auto-Mag® X-Pure Select reagents

6. Repeat step 5 for second 80% ethanol wash.

7. Keep the sample on the magnet, and air dry the magnetic beads at room temperature for 5 minutes. Remove any residue liquid with a pipettor.

8. Remove the sample from the magnet. Add 20-50µl of elution buffer and pipette mixing 20 times. Ensure beads are no longer attached to the side of the well.

9. Incubate the sample at room temperature for 5 minutes.

10. Place the sample back on the magnetic separation device and wait 5 minutes or until the magnetic beads are completely cleared from the solution.

11. Transfer the eluate containing the recovered DNA to an appropriate storage vessel and keep at -20°C for long term storage.

Customized Protocol and Programmed Procedure for Automation Purification

To obtain a custom protocol for a specific fragment size selection; or automating this procedure on a liquid handler or a magnetic processor, please contact AMD Biotech for instrument-specific instructions or additional processing procedures.

Phone: 404-290-5063 (in US), Email: support@amdbiotech.com

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact technical support via Phone: 1-404-290-5063 (in US), Email: support@amdbiotech.com

Observation	Possible Causes	Comments
Low yield/ Incorrect recovery	Highly fragmented gDNA.	Recovery will be low if DNA is not HMW. Verify the 50% of input DNA should be ⩾30 kb
	Low concentration of input DNA	Verify the input gDNA concentration using Qubit dsDNA broad range assay or equivalent. Try increasing concentration of input DNA up to the maximum of 150 ng/µl.
	Insufficient Mixing	Mixing thoroughly during the initial bind mix and elution mix is critical. Ensure the beads get sufficiently resuspended.
	Bead Loss	If beads get aspirated into tips during supernatant removal, the nucleic acid bound to these beads will also be lost. Aspirate slowly and remove as much of the first supernatant as possible without disturbing the bead.
	Handling error.	Ensure that proper care is taken with pipetting steps.
Fragment size incompatible	Insufficient ratio	Use volume ratios outlined in this manual.
	Insufficient ethanol concentration used for washing step	Use freshly prepared 80% ethanol. Over time ethanol becomes more diluted through evaporation and absorption of atmospheric water. Therefore, parts of the DNA pellet go into solution and DNA fragments are washed away.
	Elution buffer volume insufficient	Bead pellet must be covered completely with elution buffer
	Beads over dried	Do not dry beads for longer than 15 minutes at room temperature. Over drying of beads may result in lower elution efficiencies.
Downstream applications are unsuccessful	Carry-over of ethanol from washing step	Be sure to remove all the ethanol after the final wash step. Dry beads 3-10 minutes at room temperature.
Carry-over of beads	Time for magnetic separation too short	Increase separation time to allow the beads to be attracted to the magnetic pins completely

Ordering Information

Product Description	Catalog No.	Size
Auto-Mag® Mega Select	S005-01 S005-02 S005-03 S005-04	25 Preps 100 Preps 250 Preps 1000 Preps
Auto-Mag® DNA Elution Buffer	B228-01 B228-02 B228-03	50 ml 250 ml 500 ml
Auto-Mag® X-Pure Select	S005-01 S003-02 S003-03 S003-04	5 ml 50 ml 250 ml 500 ml

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